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WO9833939A1: METHOD FOR DETERMINING NUCLEIC ACID BASE SEQUENCE AND APPARATUS THEREFOR

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Abstract: A method for determining a DNA base sequence which comprises fixing in an extended state a molecule of a single-stranded sample DNA (7) carrying a bead (5) at one end thereof and a magnetic bead (6) at the other end within the visual field of a fluorescence microscope by means of a magnetic force (11) and a laser trap (3); binding a primer (8) thereto; effecting an extension reaction (10) with a polymerase to thereby cause the incorporation of a single chemically modified nucleotide (9) alone labeled with a fluorescent substance differing from base species to base species; measuring exclusively the fluorescent substance thus incorporated as a fluorescent microscopic image by the evanescent irradiation (13) with an excitation laser (1); determining the base species from the fluorescent substance; liberating the fluorescent substance with which the incorporated nucleotide has been labeled by the evanescent irradiation (13) with an ultraviolet laser (2); and then effecting the step of the incorporation of the next nucleotide followed by repeating these steps. Thus, the base sequence can be determined by using a single DNA molecule, and a DNA consisting of several hundred bases or more can be efficiently sequenced.

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Attorney, Agent, or Firm:
Foreign
References:

none

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(51) 国際特許分類 C12Q 1/68, C12M 1/00, G01N 33/50, C12N 15/10	A1	(11) 国際公開番号 WO98/33939 (43) 国際公開日 1998年8月6日(06.08.98)
(21) 国際出願番号 PCT/JP97/0239 (22) 国際出願日 1997年1月31日(91.01.97) (71) 出願人 (米国を除くすべての指定国について) 株式会社 日立製作所(HITACHI, LTD.)(JP/JP) 〒101 東京都千代田区神田駿河台四丁目6番地 Tokyo, (JP) (72) 発明者; および (75) 発明者/出願人 (米国についてのみ) 穴沢 隆(ANAZAWA, Takashi)(JP/JP) 植松千宗(UEMATSU, Chihiro)(JP/JP) 〒185 東京都国分寺市西郷ケ嶺3-8-1 Tokyo, (JP) 岡野和宣(OKANO, Kazumori)(JP/JP) 〒353 埼玉県志木市本町5-17-2-402 Saitama, (JP) 神原秀記(KAMBARA, Hideki)(JP/JP) 〒192 東京都八王子市北野台1-4-3 Tokyo, (JP) (74) 代理人 弁護士 小川勝男(OKIAWA, Katsuo) 〒100 東京都千代田区丸の内一丁目5番1号 株式会社 日立製作所内 Tokyo, (JP)		(31) 指定国 CN, JP, KR, US, 欧州特許 (AT, DE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NI, PT, SE). 送付公開書類 国際調査報告書
(54) Title: METHOD FOR DETERMINING NUCLEIC ACID BASE SEQUENCE AND APPARATUS THEREFOR (54) 発明の名称 核酸塩基配列決定法及び核酸塩基配列決定装置 (57) Abstract A method for determining a DNA base sequence which comprises fixing in an extended state a molecule of a single-stranded sample DNA (7) carrying a bead (5) at one end thereof and a magnetic bead (6) at the other end within the visual field of a fluorescence microscope by means of a magnetic force (11) and a laser trap (3); binding a primer (8) thereto; effecting an extension reaction (10) with a polymerase to thereby cause the incorporation of a single chemically modified nucleotide (9) alone labeled with a fluorescent substance differing from base species to base species; measuring exclusively the fluorescent substance thus incorporated as a fluorescent microscopic image by the evanescent irradiation (13) with an excitation laser (1); determining the base species from the fluorescent substance; liberating the fluorescent substance with which the incorporated nucleotide has been labeled by the evanescent irradiation (13) with an ultraviolet laser (2); and then effecting the step of the incorporation of the next nucleotide followed by repeating these steps. Thus, the base sequence can be		

